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Editorial

THE HEALTH OF LIVESTOCK

We have on several occasions drawn attention to the somewhat unsatisfactory condition concerning the health of livestock in this country. Announcements continue to appear in the Press that we must endeavour more and more to obtain maximum supplies of the various foodstuffs from internal sources. It has also been stated recently that the consumption of milk is increasing and that it is anticipated that it will increase further in the near future. In view of these facts it is of the greatest importance that only productive animals are retained in our flocks and herds and that every possible means are adopted to maintain them in a state of health and high productivity. The culling of dairy cows is in progress. On many farms there is room for severe culling of unhealthy and non-productive animals; in other herds culling to any great extent would be definitely uneconomic from the national point of view. At the present time members of the veterinary profession are not directly concerned with the work. Apparently there is a plan to include them on panels, if and when they are formed; the panels will visit herds and advise on the animals to be culled. This is all to the good and we welcome the use of the veterinary profession in this work. On the other hand, we are tempted to ask whether better and more rapid results might have been reached by the use of the veterinary profession at an earlier stage. We can visualise how

veterinary surgeons could have assisted stockowners and others to reach decisions concerning the animals to be removed from the herds. As disease and non-productivity are intimately related, and as some types of disease, especially if long-standing, affect the productivity of the dairy cow permanently, their recognition and elimination from the herds should be one of the first considerations in the general culling campaign. Unfortunately, some of the diseases in question are not readily recognised by stockowners: veterinary surgeons could certainly assist in their recognition. Again, some diseases, especially in the early stages, though affecting productivity, are amenable to treatment following which there is a return to normal life. To differentiate between them and old-standing disease conditions is not always a simple matter and requires the skill of experienced members of the veterinary profession. The treatment of such animals also calls for skill, judgment and experience and must be the work of the veterinary surgeon.

Without doubt, the veterinary profession can be used to a greater extent in the national effort to produce more and more foodstuffs. There are certainly indications that its services are being, and will continue to be, used to a greater extent as time goes on. But we are impatient to see the maximum effort being made and we make bold to say that this will not be attained till the Profession is brought more intimately into the various schemes for the culling of unproductive animals and the maintenance of health of those on which we are to rely for our milk and meat supply. In time of war, when "control" is one of the watchwords in a country, we may ask ourselves if some measure of "control" of the health of livestock should not find a place in the organisation of things.

Last month we congratulated the Minister of Agriculture on his decision to set up the Agricultural Improvement Council and assured him that the Veterinary Profession would respond loyally to recommendations concerning animal health and disease which that body might make. We also noted with gratification that members of the Veterinary Profession were included in the membership of the Council. It is with regret that we note that the corresponding body for Scotland contains no members of the Profession in its constitution. We are inclined to the view that this may have been an oversight of the Secretary of State for Scotland and his advisers and feel sure that this omission will be rectified at an early date. In Scotland, as in England, the health of livestock must occupy an important place in the agricultural policy.

General Articles

THE COMPARATIVE VALUE OF CERTAIN MEDIA FOR THE ISOLATION OF TUBERCLE BACILLI

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In order to determine the comparative value of media commonly employed for the primary cultivation of tubercle bacilli, cultures were made from 45 cases of human, 69 of bovine and 50 of avian tuberculosis on a series of media, viz., egg, Lowenstein's, Petragnani's, Petroff's, Sweany and Evanoff's, Herrold's, and a modification of Petragnani's medium recently described by Holth. The method of preparation of these media is described in the Appendix. With bovine strains media without glycerine alone were employed, but with human and avian strains, in addition to these, media containing 5 per cent. glycerine were also seeded. It will be observed that Holth's medium which contains tuberculin necessarily contains a certain amount of glycerine, but the amount is very small, perhaps less than 0.4 per cent. A glycerinated Holth's medium containing 5 per cent. glycerine was also prepared, as in the case of other media, for use with human and avian strains.

Technique and Preparation of Material

As it became apparent, soon after the work was started, that infected material stored at room temperature for over 24 hours, especially in the summer months, could not always be relied upon to yield pure cultures unless treated in some way, all specimens, except the first few, were treated with a 5 per cent. solution of caustic potash.

A small fragment of the material was removed aseptically from the affected gland or tissue and transferred to a short stout tube with a conical bottom, where it was thoroughly triturated with a few drops of normal saline, by means of a glass rod ground at the extremity. As the object was to obtain good growths, the amount of saline used for trituration was usually not more than four or five drops. At this stage a microscopic examination of the emulsion was carried out, and if it revealed large numbers of organisms, a slightly larger quantity of saline was added. The emulsion was then allowed to stand, the heavier particles allowed to settle down and the supernatant

fluid transferred to another tube to which was added an equal volume of a 5 per cent. solution of caustic potash. The two were then well mixed either by shaking or by means of a capillary pipette. The tube was then placed in an incubator at 37 degrees C. for 45 minutes, the contents being shaken from time to time. This amount of exposure was, in general, sufficient to kill all contaminants, but as some of the material from fowls was very old, 8-10 days, it was treated sometimes for as long as 4-5 hours without any evidence that such prolonged exposure had any detrimental effect on the bacilli. As a matter of fact, material was treated experimentally for as long as 24-48 hours without the viability of the bacilli being apparently affected. Probably the albuminous matter present in the emulsion exercises a protective action on the bacilli. It is therefore advisable where prolonged exposure is attempted, as with grossly contaminated material, to ensure that the emulsion is not too thin and contains sufficient protein material.

The specimens of human sputa were also treated with caustic potash, but in this case a few cultures, in spite of such treatment, proved to be contaminated.

Media tubes were inoculated direct with the treated emulsion and the mixture was not neutralized. With the bovine strains two tubes of each of the media were inoculated, but with the avian and human strains, one tube of each medium containing 5 per cent. glycerine was also sown. The amount of emulsion used for inoculating each tube was kept as far as possible the same—a loopful—which was well spread over the surface of the medium with the help of the water of condensation or distilled water which had been previously added. The cotton plugs were then sealed with paraffin wax and the tubes placed in the incubator at 37 degrees C. For the first fortnight, the tubes were examined daily for evidence of growth; later at intervals of two to three days.

Some of the specimens, especially those from bovines and guinea-pigs inoculated with tuberculous cow's milk, were very poor in bacilli and yielded either very few colonies rendering their early detection difficult, or only an odd colony or so was found on one medium and not on others. This made it difficult to assess whether the absence of colonies on certain media was due to the absence of tubercle bacilli in the seed material or to the unsuitability of the respective media. While such specimens had their value, for comparative purposes, only those fairly rich in bacilli were taken into consideration, so that the absence of growth on any particular medium could be definitely attributed either to the unsuitability of the medium or to some other cause. This explains why, in the tables given hereunder, the number of strains shown is sometimes fewer than those from which cultures were attempted. Occasional loss of cultures from contamination or through drying also partially accounts for this disparity.

The results of this cultural study have shown that all media are not equally valuable for the isolation of tubercle bacilli. It has been found that

strains, whether human, bovine or avian, vary widely in their capacity to multiply in an artificial medium and to produce colonies. Some have greater adaptability than others with the result that they can multiply equally well on all media and produce visible growth in about the same space of time. These are in general fast-growing strains. Others multiply more readily on some media than on others, thus producing colonies on different media after different periods of incubation. It is in reference to these strains that the special characteristics of a medium are best seen. Since these are to some extent related to the type of the bacillus concerned a detailed account of the relative merits of the different media is given separately under the three types of tubercle bacilli.

Human Type

The total number of strains studied was 45, all isolated from sputa supplied by Dr. Pryce, of the County Sanatorium, Harefield, Middlesex.

The specimens were, in general, strongly positive, some on smear preparation appearing like pure cultures of tubercle bacilli. The sputa were treated with 5 per cent. caustic potash before being cultured, but in view of the large number of contaminants, the material being sometimes several days old, a few cultures proved to be contaminated.

Lowenstein's medium proved superior to others for the primary cultivation of human tubercle bacilli. Colonies on this medium appeared earlier than on any of the others; they appeared more or less after a fixed period of incubation, and because of the smooth, green background of the medium were easily seen. If glycerine was included in the medium, in about two weeks or so the growth was profuse even before colonies had appeared on other media. Contamination was infrequent and, if it occurred, was confined to a small patch so that pure cultures could almost always be recovered from the clean uncontaminated portion. There was no evidence that the dye contained in the medium had any inhibitory effect on the bacilli; as a matter of fact, with material poor in bacilli, more colonies were seen to develop on Lowenstein's than on egg or other media. Different batches of media prepared with ordinary care were remarkably consistent in quality. This was not the case with egg and some other media like those of Herrold, Sweany and Evanoff, and Holth, which for no obvious reason showed very wide variations in this respect.

The following table demonstrates the superiority of Lowenstein's medium over the egg medium from the point of view of the date of appearance of the colonies. The egg medium has been especially chosen for this comparison because in virtue of its ease of preparation it is the one most commonly employed for the cultivation of the tubercle bacillus; also because on none of the other media did the growth appear earlier than on Lowenstein's.

In seven other cases the tubes were not examined until the thirteenth day, by which time growth had appeared in every case on Lowenstein's medium but not on egg.

XUM

Period of incubation. (in days)	*Lowenstein's medium.*	*Egg.*
8	7	0
9	10	0
10	9	0
11	2	6
12	1	2
13	2	3
14	0	2
15	0	9
16	0	3
17	0	3
18	0	2
19	0	0
20	0	1
	31	31

The majority of the strains showed growth on Lowenstein's medium at about the eighth to tenth day and in general by the eleventh to twelfth. Most strains had produced colonies. On the egg medium growth appeared on the average about the fifteenth day and sometimes not until much later.

Petragnani's and Petroff's media were found to be almost as good as Lowenstein's, but growth appeared later. Petragnani's medium has the additional disadvantage that it is rather more difficult to prepare and lacks the clear smooth surface of Lowenstein's medium, so that colonies when they first appear are not so easily seen.

Sweany and Evanoff's medium proved unreliable; sometimes no growth appeared at all, or it was poor. Some strains, however, grew very well on this medium, producing large, warty colonies and, if glycerine was present, a thick greyish-white wrinkled layer.

Herrold's medium also was not so reliable as Lowenstein's. Some strains did not grow at all; others produced colonies late, sometimes after several weeks' incubation. Moreover, they were seen to arise in an irregular manner, being few at first, becoming more numerous later on. The medium tends to dry up quickly unless proper care is taken.

The same applies to Holth's medium. While the majority of strains grew well, others produced no growth at all or only a few colonies. However, when good growth occurred it was profuse, more profuse even than on other glycerinated media. The colonies coalesced to produce a dry, warty layer with a characteristic brownish to deep reddish pigment. In fact, the number of strains that produced this pigment was remarkable considering that most human strains rarely produce a pigment in primary culture on the egg medium.

Strains which failed to produce pigment in primary culture usually did so in subculture. There appears to be something in this medium which is especially conducive to this pigment formation. Were it not for the fact that certain batches of media proved unreliable or that certain strains failed to grow, this medium would appear to be the most suitable for the isolation of the human tubercle bacillus, for though the growth appears more slowly than on Lowenstein's medium, it is profuse and has a very characteristic appearance. The addition of glycerine to the medium improves the growth still further. Incidentally, in subculture most strains produced a luxuriant growth in four or five days, very often with the usual pigment formation.

Bovine Type

Cultures were attempted from 69 cases in all, in 22 from gland material from infected bovines, and in 47 from the inguinal glands of guinea-pigs injected with tuberculous cow's milk, but as some of the material on microscopic examination showed only few or no bacilli, the total number of cultures available for exact comparison was small.

From the point of view of the date of appearance of the colonies and reliability and consistency of results, Lowenstein's medium was found to be superior to all the others. Colonies on this medium usually appeared about the 7th-13th day and had become prominent even before they appeared on other media, though with bovine strains there was not a fixed time interval as was found to be the case with human or, as will be seen later, with avian strains. Different batches gave very consistent results; in no case where microscopic examination had revealed the presence of bacilli did cultures fail. With material very poor in bacilli, colonies were sometimes seen on Lowenstein's medium but not on others. With regard to the subsequent rate of growth, however, Lowenstein's medium showed no superiority over the others as, after a time, the growth on all media, especially Lowenstein's, Petragnani's and egg, was found to be almost equally good.

The following table shows the rate at which growth appeared on egg and Lowenstein's medium in the case of 36 strains. For the reasons explained under the human type, other media have been excluded.

Other media proved less reliable. Certain batches of Herrold's showed no growth at all. Moreover, the colonies on this medium appeared more tardily, being very few at first, later becoming more numerous. The colonies, however, had a rather characteristic shiny appearance.

The same applies to Sweany and Evanoff's medium. Isolated colonies on this medium, however, sometimes grew to a very large size.

Petroff's medium was found to be quite good, while Holth's proved very unreliable, the majority of strains not growing at all or producing only a few isolated colonies. Those that grew well produced profuse growth, superior to that on other media.

XUM

Period of incubation. (in days)	*Lowenstein's medium.*	*Egg.*
7	4	0
8	3	0
9	3	0
10	12	2
11	3	5
12	4	2
13	4	4
14	1	3
15	2	2
16	0	1
17	0	2
18	0	3
19	0	4
20	0	1
21	0	3
22	0	2
23	0	1
	36	36

Summarising, it may be stated that no medium seemed to have any outstanding advantages for the isolation of bovine tubercle bacilli. Lowenstein's medium is, however, preferable to others in view of its reliability, and the fact that it shows up the growth earliest, besides possessing other advantages which have been enumerated under the human type.

Avian Type

Cultures were made from 50 cases in all of tuberculosis in fowls, the material used being either the spleen or the liver. Most of the material was in an extremely decomposed condition, having been collected from fowls dead over a week or more and kept unopened at room temperature; still, no difficulty was experienced in obtaining pure cultures in every case following the caustic potash technique described above. The possible explanation may be that many of the contaminants seen in such material were of the non-sporulating type, such as streptococci, staphylococci and non-sporulating bacilli which were presumably easily killed off by the caustic potash.

The specimens were very rich in bacilli and yielded a very large number of colonies. Of the 50 specimens collected, cultures were obtained in every case. This contrasts with bovine or guinea-pig material, which was sometimes

very poor in bacilli, and on cultural examination yielded no growth or but a few colonies.

All the 50 strains grew with ease on all the media; also, the number of colonies developing on the different media was approximately the same. Some of the media belonged to batches on which the human and bovine bacilli had failed to grow; this shows that the avian type of bacillus is less exacting in its requirements for growth than either the human or bovine type.

When, however, the date of appearance of the colonies was considered, it was found that colonies of the avian tubercle bacillus appeared earliest on Lowenstein's medium, sometimes as early as the fifth day. The majority appeared about the sixth or seventh day, but in every case growth had appeared by the ninth day.

Also, as noted under other types, strains of avian tubercle bacilli showed marked variation with regard to the rate at which they multiplied and produced colonies in primary culture on the different media. Some fast-growing strains produced colonies on all the media on about the same day, but with other strains colonies were seen on different media at different times.

The following table shows the date of appearance of colonies on Lowenstein's and egg media. The number of strains shown under the egg medium is less than under Lowenstein's for the reason that, with the remaining strains, colonies appeared later than the 15th day. On most media colonies had usually appeared by the 15th day, but with some strains growth was not seen until the 21st day.

Period of incubation. (in days)	*Lowenstein's medium.*	*Egg.*
5	13	1
6	20	2
7	10	5
8	3	10
9	4	5
10	0	4
11	0	4
12	0	2
13	0	3
14	0	1
15	0	1
	50	38

While, therefore, Lowenstein's medium was found to be superior to others in that it showed up the growth earliest, the subsequent growth was not equally

rapid and after a few days' incubation the growth on the other media became as good as on this medium. This refers only to primary colonies.

Up to about the third week there was no difference between plain and glycerinated media with regard to the amount of growth. Thereafter the colonies on glycerinated media began to grow to a larger size and this continued for several weeks. The colonies very often coalesced, producing a moist, sticky layer, which was usually creamy-white to creamy-yellow on egg and on the media of Holth and Sweany and Evanoff, and light brown on those of Lowenstein and Petragnani.

Another noteworthy point in connection with these cultures was that though the avian bacillus grew with relative ease on all the media and colonies became visible earlier than was the case with human or bovine strains, the subsequent growth was very slow. For periods up to 6-8 weeks the colonies on plain media were nothing more than tiny specks and only just visible to the naked eye. In this respect the avian bacillus appeared to be the slowest-growing of the three types.

After about the fourth week secondary colonies began to appear and continued to do so for 10-12 weeks. Some strains produced secondary colonies more readily than others. Secondary colonies were either spherical, hemispherical or umbilicated. They arose with the greatest frequency on Petragnani's medium, less so on Lowenstein's, but they were seen on all. They occurred with the least frequency on Herrold's medium. The same strain produced rounded or hemispherical colonies on one medium, both rounded and umbilicated on another. Usually all types of colonies were seen on the same medium, though the umbilicated were always in a minority. Secondary colonies, since they appeared at different times, showed wide variations in size on the same medium.

With regard to the abundance or luxuriance of growth, Holth's medium proved superior to all others, the colonies coalescing to form a thick, very moist, sticky layer with a creamy-white to creamy-yellow or even a light pinkish tinge. The glycerinated medium showed even better growth.

Conclusion

Lowenstein's medium is superior to the others used in this series for the isolation of tubercle bacilli. With all types growth appears earliest on this medium, the human strains producing visible colonies in 8-12 days and avian in 5-7 days. Colonies of bovine strains do not appear after a fixed period of incubation, as is the case with human and avian strains, but they appear earlier than on the other media tested. Both human and avian strains grow well on this medium, and the bovine no worse than on the others.

The medium is very reliable. Different batches of medium are remarkably consistent in quality and can always be relied upon for the isolation of a culture from any material in which the presence of tubercle bacilli is suspected.

The medium offers a smooth green background and colonies are easily seen as soon as they have appeared.

Contamination is infrequent and if it occurs is usually confined to a small area of the surface of the medium.

There is no evidence that the dye contained in the medium has any inhibitory effect on the bacilli.

While cultural characteristics alone cannot always be relied upon for the typing of tubercle bacilli, in view of the early appearance of the colonies this medium enables a rough classification of an unknown strain to be made in a shorter space of time than other media.

Here a word may be said about Holth's medium. Though colonies appeared later than on the medium of Lowenstein, the ultimate growth produced on this medium with each of the strains was superior to that on other media. It was also most characteristically differentiated, the human strains producing large, dry, warty colonies with a brownish to deep reddish pigment, the bovine, thin grey flat colonies with raised centres, and the avian, creamy-white, moist, shiny colonies which coalesced, forming a very moist, shiny, sticky layer, sometimes with a light pinkish tinge. On the glycerinated medium the growth of the human and avian types was even more profuse. In subculture all strains grew very well, producing good growths in five or six days. Even old stock strains kept in artificial cultivation for long periods did not show such abundant growth on the other media. It was therefore very disappointing to find that some human and bovine strains failed to grow or grew very poorly on this medium.

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APPENDIX

Preparation of the different media

(1) *Plain egg.*

Egg pulp, 2 parts.
Normal saline, 1 part.

Mix thoroughly, strain through muslin. Inspissate in the serum inspissator at 75 degrees C. and sterilise by heating in the inspissator at 75 degrees C. for two hours on three successive days.

(2) *Petragnani's medium.*

Fresh milk, 300 c.c.
Peptone, 2 gms.
Potato meal (B.D.H.), 12 gms.
Finely grated potatoes, 2 (small).

XUM

Mix in a beaker and place the beaker in a water bath at 100 degrees C., stirring the mixture constantly for 10 minutes. Allow the beaker to remain in the water bath for 1 hour; then remove and cool to 60 degrees C. Now add 8 whole eggs, 2 egg yolks, and 20 c.c. of a 2 per cent. solution of malachite green. Mix well, filter through muslin and tube. Slant and coagulate the medium at 90 degrees C. for 1 hour; sterilise by inspissation at 80 degrees C. for 20 minutes on two successive days.

(3) *Holth's medium* (modified Petragnani).

Egg pulp, 90 c.c.
Potato juice, 60 c.c.
Tuberculin, 3 c.c.
Lactic acid 5 per cent., or hydrochloric acid, 5 c.c.
Potato juice.
Potato mash, 500 gms.
Distilled water, 1,000 c.c.

Cook at 115 degrees once and filter. Sterilise filtrate in the steamer on two days or at 115 degrees once. Add to the egg pulp as above.

(4) *Lowenstein's medium.*

(1) Potassium dihydrogen phosphate, KH_2PO_4, (Analar), 0.4 gm.
Magnesium sulphate (Analar), 0.04 gm.
Magnesium citrate, 0.1 gm.
Asparagin, 0.6 gm.
Distilled water, 100 c.c.

Heat to dissolve. Place in the steamer for two hours and allow to cool overnight.

(2) To each 600 c.c. of (1) add 30 gms. of potato starch. Mix and heat in a water bath with constant stirring for 15 to 20 minutes until a satisfactory paste is produced, and allow to remain for one hour in the water bath at 56 degrees C.

(3) To each 600 c.c. of above now add 1,000 c.c. of egg pulp, previously filtered through sterile muslin, and 20 c.c. of 2 per cent. malachite green.

Slant and coagulate the medium at 90 degrees C. for 1 hour; sterilise at 80 degrees C. for 20 minutes on two successive days.

(5) *Herrold's medium.*

Lab. Lemco, 3 gms.
Peptone, 10 gms.
Sodium chloride, 5 gms.
Distilled water, 1,000 c.c.

To this add 2 per cent. agar and adjust to pH 7.5. To 150 c.c. of this nutrient agar, which is cooled to 55 degrees C., add one egg yolk. Shake well

to ensure thorough mixing and strain through sterile muslin. Tube and allow to set in a sloping position.

(6) *Petroff's medium.*

Finely ground-up veal, 1 lb.
Distilled water, 500 c.c.

Mix and allow to stand for 48 hours in the ice chest, with occasional shaking. Filter through sterile muslin, and to the filtrate add double the amount of fresh, well-beaten eggs. Mix thoroughly and add 1 per cent. gentian violet in 95 per cent. alcohol to make a final dilution of 1 : 10,000. Tube and inspissate at 95 degrees C. for 1½ hours. Sterilise by inspissation for 1 hour at 75 degrees C. on three successive days.

(7) *Sweany and Evanoff's medium.*

Finely ground-up veal, 450 gm.
Milk, 450 c.c.

Allow to stand in the ice chest for 48 hours with occasional shaking. Filter through sterile muslin and to the filtrate add double the amount of fresh well-beaten eggs. Stir well, strain through muslin, tube and coagulate for 1 hour at 90 degrees C. Sterilise by inspissation for 1 hour at 75 degrees C. on three successive days.

N.B.—In the preparation both of Petroff's medium as well as Sweany and Evanoff's, fresh veal was used instead of sterile dried veal.

RHODODENDRON "POISONING" IN CATTLE

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FEBRUARY 13 and 14, 1941, in this neighbourhood were marked by severe rain and snow storms. During the night of these dates a bunch of eight-yearling cattle and one aged cow, seeking shelter from the weather, found their way into a plantation of rhododendron bushes, and had, from all appearances, a "good feed" of the leaves.

General symptoms.—Animals very depressed and disinclined to move—head held low—salivation—tympany—constipation (not diarrhœa, as mentioned by Lander)—severe straining but no expulsion of fæces, only a little mucus—shivering—inco-ordination of movement in all limbs, but especially of the hind limbs—staggering gait—eventually decubitus and extreme difficulty in regaining an upright position. In several of the animals the act of lifting the head to

drench them caused them to fall—there was no struggling when down. In all the animals vomition was frequent and consisted mainly of rhododendron leaves—some partly masticated—some whole.

Considering the symptoms, i.e., salivation, straining and no passage of fæces, I came to the conclusion that exosmotic action and a rapid peristalsis were indicated. I therefore gave each animal 4 c.c. carbymecholine. This resulted in greatly increased salivation and an action of the bowels, at *first* fluid and then hard, dark brown, caked fæces. Owing, I think, to the increased *out*flow of fluid into the rumen there was also increased vomition. At any rate, that is how I regarded it, because physiologically the action of the rumen is a peristalsis, but a peristalsis in the opposite direction to peristalsis of the bowels, i.e., towards the mouth.

This treatment was followed by linseed oil 4℥ + ½℥ chlorodyne twice a day.

There was a gradual improvement in most of the cattle after the first three days, when rumination commenced. This was followed by increased appetite and on February 20 all were convalescent.

Extraordinary appearances.—By this I mean departure from the ordinary symptoms as shown in the majority of the cases.

1. The old cow.—This animal must have eaten a large quantity of the leaves. She was very excited if anyone approached her, and the muscular spasms "jerked" her off her feet; for a considerable time she was unable to stand. She filled the manger with vomit and saliva after a dose of the choline, and passed an enormous amount of fæces. Her milk dried up, but she was practically normal in three days.

2. Eighteen months old Hereford bullock (castrated male).—This animal, too, must have made a good meal of the leaves. He was very depressed, salivation very marked—very constipated, and vomition was persistent. I gave this animal three doses of choline in three days in addition to the oil and chlorodyne, before there was any bowel movement. On the fourth day he "cudded," ate hay and drank water. He made an "uninterrupted recovery."

3. Red heifer about a year old.—This animal was unable to walk the two miles home and was pulled into a nearby shed. No vomition or salivation. Rumen very bloated. Constipation serious. She was more or less comatose. Gave two doses choline in addition to the oil, etc., but with no result. Then I gave half an ounce "purgo" and even this had no result for 48 hours. She was "down" for six days but after the evacuation of the bowels she was soon on her feet and recovered fairly quickly.

"*Active principle.*—The leaves contain a *tannin,* resolved by *acids* to rhodoxanthine. The leaves also contain a *bitter resin* and contain the active principle andromedotoxin."—(*Lander.*)

Tannin would most likely account for the constipation, which probably occurred in the second and third stomachs, as I suppose there would not be

much acidity until the fourth stomach was reached. It is therefore improbable that *rhodoxanthine*, whatever its action may be, was present at this stage.

Probably also the symptoms apart from vomition may be due to the toxin—andromedotoxin.

This is of course my own opinion, but it seems quite feasible, and may be put forward as *a* cause, as the chemistry of rhododendron poisoning is not yet complete.

THE BEARING OF A SECOND LITTER BY CERTAIN RODENTS WITHOUT MATING AFTER THE FIRST PARTURITION

By HAROLD BURROWS

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THREE years ago the writer observed a mouse which bore a second litter while nursing a family 21 days old. The mouse, which belonged to the inbred Murray-Little *dba* strain, had been caged with a littermate brother until found to be pregnant; thence onward she was isolated from any mouse but her own young. No record was made of the date of isolation, but it was before parturition. I had effected the isolation myself, and when the second litter appeared it was felt that someone had allowed the access of a male during the postparturitional œstrus. In the particular circumstances this seemed most unlikely, but at that time I was not prepared to accept any other explanation. Since 1938 breeding has been in progress at this institute from two pure strains, namely Wistar rats and *dba* mice, and brother-sister mating alone has been allowed. Our consistent practice with the rats has been to keep each littermate pair together until the female is manifestly pregnant, when she is transferred to a separate cage and remains there until the young are taken away. Usually the female has been isolated 48 hours or so before delivery. Owing to lack of accommodation this procedure was not possible as a routine with the mice, but was carried out from time to time when conditions were favourable. With most of the mice we have not recorded how often isolation before parturition was practised and so can offer no figures to denote the percentage frequency with which, in these animals, second litters may be born in the absence of postparturitional mating. But in one experiment which included 125 females our purpose was to allow a single litter only to each mouse, and isolation before delivery was strictly carried out; in this series the

phenomenon occurred once only, that is to say, in 0.8 per cent. of the total pregnancies.

In December, 1938, Mr. Smith, who has charge of our breeding rats, reported that one had borne a second litter although she had been isolated since the recognition of her first pregnancy. Thereafter a watch was kept by Mr. Ewers and Mr. Smith on our breeding rats and mice for any recurrence of the same event; as a result we now have records of ten rats and two mice which have borne second litters in the absence of mating after the first parturition (Table I). The ten examples in rats occurred amongst a total of 210 breeding does, and, as shown in the table, happened, with one exception, in the spring. During the period under review these inbred Wistar rats produced 473 litters, so that the phenomenon has now been recorded in just over two per cent. of the total pregnancies among them.

In 1940 we took additional precautions against the unintended access of a male by binding the lids to the boxes which contained isolated pregnant females. Later it was suggested to the writer that this precaution was inadequate because copulation might take place through the bars of the cage lid. Though this appears impossible with our cages, to meet the objection a second lid was applied in such a way as to eliminate the hypothetical chance of coitus through the bars. Even with this safeguard a female (No. 10 of the table) bore a second litter without any coitus having followed the earlier parturition. Since that date we have had no additional examples, but we have had little opportunity for further inquiry; our breeding establishment was closed for a while and, though open again now, is on a very small scale.

When mentioning the observations here recorded to other laboratory workers, it has been learned that they too have come across instances in which second litters have been borne in the absence of any known opportunity for postparturitional mating. But the general tendency has been to assume, as I did at first, that some overlooked opportunity must have been given for mating after the birth of the first litter. Our own experience suggests that such an assumption is unjustified.

Occasional instances of the anomaly now under review can be found in the literature. Du Boisson (1939) mentions two examples in mice; before parturition and thereafter the pregnant mothers were isolated and yet each bore a second litter 21 days after the first. In these examples the young of the first litters numbered three in one case and four in the other. The parents were of a Dutch black and white strain and the young of the second litters accorded with that strain. Pickard (1928) refers to a case reported to him as having occurred in a rabbit. A Havana doe had been bought in April and was mated on the 24th of that month, after which she was kept isolated. On May 1 she had a litter of six which died within a week. On May 24, thirty-one days after the mating, she had another litter of six. The young of both litters

appeared normal at birth. Evidently the rabbit had been pregnant when bought, though this was unknown to the purchaser, and the birth of the second litter indicates that fertile copulation had occurred during pregnancy.

Discussion

If the observations we have recorded are correct, they must be attributed to two events, namely: (1) copulation during pregnancy, and (2) a survival of the spermatozoa long enough to render the coitus fertile. Before considering these two points, reference may be made to the fact that in rabbits, rats and mice œstrus supervenes and coitus is permitted within 48 hours after parturition. This is the postparturitional œstrus mentioned in the text.

1. *Copulation during pregnancy.*—Hammond and Marshall (1925) have shown that in the rabbit pregnancy is not a bar to coitus; after the 14th day of gestation, coitus, they say, will almost always be allowed. In the rat also Long and Evans (1922) noted that copulation is not inevitably abolished by pregnancy, and Nelson (1929) has made the same observation. Nelson detected regular œstrus cycles, as shown by vaginal smears, in a pregnant rat which permitted coitus at each recurring cycle. Œstrus and copulation were observed in this rat on the 5th, 9th, 13th, 17th and 21st days of pregnancy.

2. *Prolonged viability of spermatozoa.*—In the examples of pregnancy without postparturitional mating quoted above it seems necessary to suppose that, in the particular conditions, the spermatozoa survived for a period which exceeds the normal limits of their viability in the female as determined by experiment. In the case of the rabbit reported to Dr. Pickard it would be necessary to assume, if the details as given to him are correct, that the spermatozoa retained their potency for six days after insemination whereas it has been shown experimentally by Hammond and Asdell (1926) that the normal limit of spermatozoal survival in the female rabbit is about 30 hours. The same authors found that the rabbit's spermatozoa when retained in the epididymis may preserve their fertilizing potency for 38 days.

The table shows that with our rats the interval between the two litters averaged 24.4 days; this period corresponds approximately with the normal gestation period of about 23 days, plus the time elapsing between parturition and postparturitional œstrus. Seeing that the females were separated from the males two days before parturition, the spermatozoa must have retained their fertility for about four days after insemination. In normal conditions it has been shown that with most mammals in which the matter has been investigated, including the mouse and rat, the spermatozoa become inactivated within 24 hours of their introduction into the female; and unless our own observations are wrong, some special condition must be identified to account for the extended fertility of spermatozoa in our animals. (a) In all the cases we have mentioned the first litter was small enough to have been accommodated

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in a single horn of the uterus, and (b) possibly an empty uterine horn during gestation may be a favourable habitat for spermatozoa. That pregnancy may favour the life of spermatozoa is perhaps indicated by an experiment done by Turner (1938), who observed the effect of grafting a testis from a new-born rat into the eye of a female host. He used Wistar rats and took the presence of sperm heads in the grafted testes as evidence of spermatogenesis; they were present in 6.56 per cent. of the grafts in non-pregnant rats, in 62.07 per cent. of those in spayed rats and in 44 per cent. of the grafts in pregnant or lactating rats. The numbers suggest that some factor inimical to spermatozoa is more potent in the genital tract of the non-pregnant than in that of the pregnant rat. (c) Another point is that our own instances all happened among pure stocks of inbred animals. In our rats and mice brother-sister matings only have been permitted; the animals are approximately homozygotic, and various tissues may be successfully transferred from one to the other of a pair. It seems possible that this compatibility of tissues between the mates may have led to an abnormally long survival of spermatozoa in the uterus or oviduct of the female.

TABLE I

THE BEARING OF SECOND LITTERS BY RATS AND MICE IN THE ABSENCE OF MATING AFTER THE FIRST PARTURITION

Rats	*Date of first litter*	*Number of young*	*Date of second litter*	*Number of young*	*Interval between litters (days)*	*Date of isolation*	*Date between isolation and second litter*
1	21.11.38	5	14.12.38	5	23	—	—
2*	20.2.39	5	20.3.39	6	28	—	—
3	28.2.39	7	25.3.39	11	25	—	—
4	1.3.39	5	23.3.39	7	22	—	—
5	17.4.39	2	8.5.39	3	21	—	—
6	19.4.39	6	11.5.39	8	22	17.4.39	24
7	28.4.39	6	23.5.39	4	25	26.4.39	27
8	24.3.40	7	19.4.40	8	26	22.3.40	28
9	27.3.40	6	23.4.40	8	27	25.3.40	29
10	14.2.40	6	11.3.40	5	25	12.2.40	27
Average		5.5		6.5	24.4		27
Mice							
1	31.10.38	2	22.11.38	5	22	—	—
2	17.4.39	2	8.5.39	3	21	—	—
Average		2		4	21.5		

* Same rat as No. 1.

Summary

Instances are given in which certain rodents of inbred strains have produced a second litter in the absence of intermediate mating. The occurrence, it is thought, might be attributed to: (1) copulation during the first pregnancy, and (2) a prolonged viability of spermatozoa in the unusual circum-

stances which include pregnancy confined to one uterine horn and the close genic relationship of the mates. In every case the first litter has been small enough for accommodation in a single uterine horn. It is suggested that in these conditions conception as a result of coitus before delivery may be possible at the œstrum which immediately follows parturition.

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ECONOMY IN THE USE OF SPIRIT

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THE attention of veterinary surgeons and others is drawn to the urgent necessity for strict economy in the use of alcohol in any form, whether in research or other laboratories, in teaching institutions and in general veterinary practice.

While it is fully recognised that spirit may be essential for many purposes, it is hoped that this necessity for strict economy shall be constantly borne in mind.

Considerable amounts of alcohol or alcoholic preparations are used in general veterinary practice for internal medication. Much of this can be saved without prejudice to the patient, and veterinary surgeons are asked to consider the use wherever possible of some alternative, such as ammonia preparations, for alcohol as a diffusible stimulant, and watery solutions instead of alcohol for the preparation of "tinctures" from extracts. Similarly, alcohol can be frequently dispensed with for external applications, and this should be done wherever possible.

Attention is drawn to the following abstract from a statement issued by The War Wounds Committee of the Medical Research Council:—

"The use of spirit is an unreliable method of sterilising instruments, and should be abandoned. Dry heat, where available, is the best method for most instruments composed only of steel or glass; the instruments should be sterilised in dust-proof containers. Failing the dry heat method, every instrument suitable for boiling should be sterilised by that means *immediately before use.* Syringe needles should be boiled. It has been confirmed that boiling in two

per cent. sodium carbonate for five minutes is lethal to all types of bacteria, including spores. Boiling for two minutes is adequate for most purposes, but wherever spores may be present—as after an operation for gas gangrene—boiling should be continued for fully five minutes. Moreover, recent tests, as well as the experience of many surgeons, have indicated that the widespread view that boiling blunts the edges of surgical knives has little or no foundation. It is important, however, to protect the edge from mechanical damage, by contact with other instruments, during boiling; this can be done by the use of a rack to hold knives during boiling, or by wrapping the knives in gauze or lint before they are placed in the steriliser.

"The use of spirit for *storing instruments after sterilisation* should be drastically curtailed. Where boiling immediately before use is likely to be impracticable, the sterilised instruments should whenever possible be kept in the dry state in a covered sterile receptacle or sterile towels. Where this cannot be done, *Liquor Formaldehydi Chirurgicalis* is suitable for the storage of most instruments; it consists of Borax 1.5, Solution of Formaldehyde 2.5, Phenol 0.4, Distilled Water to 100; the borax completely prevents rusting of steel instruments kept in the solution for a week or more. Instruments kept in this solution need to be rinsed before use, and, because of its formalin content and alkalinity (pH over 9), the solution is not recommended for the storage of hypodermic syringes or needles. For syringes and needles likely to be required for urgent use, storage in small quantities of spirit may still be permissible, but as a general rule syringes and needles (if of rustless steel) should be kept in the dry state after boiling. Glass instruments such as thermometers may be kept in a 1 per cent. sodium hypochlorite solution (in a covered container), or in 1 per cent. phenol.

"The use of spirit for the preparation of the surgeon's hands before operation can be avoided altogether if, after washing with soap and water for three minutes, the hands are dried on a small sterile towel before the gloves are put on; a stock of these towels should be kept in the same sterile drum as the gloves. Since the chief use of spirit is for drying the hands, and the alternative process certainly takes longer, a good supply of French chalk must be provided for each pair of gloves. The practice of keeping a bowl of spirit for the surgeon to rinse his hands in during an operation should be prohibited. If spirit is used at all for drying the surgeon's hands, it should be applied economically by means of a dropper or spray.

"The use of alcoholic solutions—e.g., tincture of iodine—for the preparation of the patient's skin for operation incision can be avoided altogether. The following alternative technique is recommended, the antiseptics mentioned having been subjected to practical trial against bacteria on the living human skin: (a) the area of the operation is washed thoroughly with soap and water, using a gauze swab; (b) the area of the operation is dried with another

gauze swab; (c) the area is painted with *Liquor Antisepticus* N.F.* or with a watery solution of either iodine (two per cent. in solution of sodium iodide two per cent.), or a solution containing brilliant green (0.5 per cent.) and crystal violet (0.5 per cent.). It is essential to delay for at least three minutes before making the incision, so as to allow time for the antiseptic solution to act, and to dry on the skin.

"The custom of swabbing spirit over the suture line when stitches are removed after clean operations should be abandoned for the duration of the war. *Liquor Antisepticus* N.F.* may be used instead for this purpose. As an alternative to a liquid antiseptic, sulphanilamide powder may be applied."

ARTHRITIS IN HORSES

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THE object of the work recorded in this article is to ascertain the infections, if any, present in cases of arthritis in horses in Egypt and to examine the value of vaccines as therapeutic agents in treatment of the condition.

Literature.—Hole and Purchase (1931) isolated *staphylococcus pyogenes aureus* from the blood of pheasants six to ten weeks old which showed evidence of arthritis; in one case staphylococcus pyogenes citreus was isolated. The disease was reproduced on inoculation of the isolated organisms into healthy young birds. Dead cultures killed by heat in two doses failed to immunize. Infection via abrasion or wound is regarded as the most probable route. The Department of Agriculture, New South Wales (1931), records two forms of arthritis in lambs and young sheep, a non-suppurant specific infection and a non-specific type associated with pyogenic organisms; an organism similar to *C. pyogenes* appears to be responsible for the specific type. Jungheer (1933) isolated *staphylococcus pyogenes aureus* from four seven months old bronze turkeys. Each of the four birds showed varying degrees of lameness and enlarged tibiometatarsal joints. The British Medical Association Committee (1933) reported that certain forms of arthritis in animals are true cases of osteo-arthritis; suffraginitis is quoted as an example. Some constitutional factor appears to encourage bone formation under the slightest stimulus, such as strain or repeated minor injury. Osteo-arthritis in horses is often accom-

* Obtainable from any wholesale drug house; or one of its proprietary equivalents as listed on pp. 30-37 of the *National Formulary for National Health Insurance Purposes*, Third Edition, 1939, may be used.

panied by peripheral interstitial neuritis. Treatment recommended in human beings include hot-packs, cautery, exercise, massage, tenotomy and deep X-ray treatment. The Committee states that further research into the bacteriology of the disease is necessary. From the serous cavities, joints, cerebral ventricular fluid and liver (not from spleen or blood or kidneys) of six piglets between five and twelve weeks of age, Mayerhoffer (1935) obtained a Gram negative, non-acid fast bacillus that required enriched media for isolation. The piglets showed lameness after a long journey by rail or road. Mayerhoffer failed to reproduce the disease (fibrinous inflammation of serous membranes and joints) by subcutaneous inoculation of this bacillus into two piglets. Buitenhuis (1935) believes that the *swine erysipelas bacillus* is the specific cause in all cases of polyarthritis in pigs. In 87 per cent. of cases examined large swellings of deep inguinal lymphatic glands were found and *B. erysipelatis suis* was isolated. Bulow (1936) isolated *Br. abortus* from a local arthritis of the hock joint in a horse. Stafseth, Thompson and Lisa (1937) isolated hæmolytic streptococci from some of the seventy-three dogs which they examined. They were recovered from numerous sites in dead puppies thought to have died from an association of "acid milk," as well as from pus in post-vaccination abscesses in older dogs; their natural habitat appeared to be the genital tract of females. The authors also describe two cases of articular rheumatism successfully treated with vaccine prepared from, *inter alia*, a hæmolytic streptococcus. Wernery (1937) states that arthritis in pigs is due to *B. erysipelatis suis* and is easily demonstrable in the joint fluid. Barry (1937) described arthritis in lambs as caused by the swine erysipelas bacillus. Murane (1938) isolated the swine erysipelas bacillus from lambs prior to and commonly after castration and docking, in which there were present lameness and deformity of limb joints with erosion of articular surfaces in chronic forms. The organism reproduced the disease in lambs when given by mouth or when dropped into the eye and was not pathogenic to pigs. Hutyra and Marek (1926), referring to articular rheumatism, state that bacterial investigation showed that streptococci were most often present in the exudate from the joints or in endocardial stratification, as well as in mucus from the tonsils. Intravenous injections of broth cultures of these streptococci into rabbits caused a polyarthritis within a few weeks. Serum horses injected with cultures of streptococci became readily affected with polyarthritis (Poels). Improper keeping of the animals, especially feeding on watery food of little food value, appears to exercise a predisposing effect. A much more common influence in this direction is exercised by cold damp air and draughts, especially if they strike the animal in a warm place. The disease occurs especially after parturition or retention of the afterbirth. Medical Research Council System of Bacteriology (1929) under the heading of infection of bones and joints reports: "While acute suppurative periostitis and osteomyelitis are usually due to staphylococcus

aureus, cases are occasionally noted in which hæmolytic streptococci are the associated organisms." Topley and Wilson (1936) report: (1) *Streptothrix muris ratti* intravenously inoculated into rabbits may sometimes lead to arthritis, and occurs as a natural disease among mice, sporadic or epidemic-septicæmic or a polyarticular type. The mortality is high; infection via bites or contamination with urine which may contain the organism. Diagnosis is made by culture. (2) Swine erysipelas bacilli have been found to be responsible for outbreaks of polyarthritis in pigs, sheep and occasionally in cattle and turkeys. (3) Acute osteomyelitis is almost always due to infection with staphylococcus aureus, occurring in children after trauma. The infection is blood-borne. In animals, other bacteria are responsible for this condition as, for instance, the diphtheroid bacillus, *C. pyogenes*. (4) The present position on the bacteriology of rheumatic infections is focused on the relation of hæmolytic streptococci and on the possibility that this disease is caused by a filtrable virus. Wooldridge (1934) includes under "Rheumatics" as the predisposing causes, climate, dampness, cold-humidity, lack of sunlight, sudden changes of temperature, draught, constipating food, the existence of other affections such as cystitis, colitis and metritis, age (the young being more subjected to acute rheumatics and the old to the chronic form); no immunity follows an attack, but one attack predisposes to subsequent attacks. No statement was made as to possible cases of bacterial origin.

Experimental

Two sets of animals were dealt with:—

I. Race Horses; and II. Cairo S.P.C.A. Horses.

I. *Race Horses.*

Seventeen cases have been examined up to date. In seven cases there occurred a sudden acute form and in the other ten a sub-acute form.

(a) The acute form sets in rapidly. The horse is usually left more or less normal at night and next morning it is found unable to move. If forced to move, it evinces signs of pain and general stiffness—the joints are painful when touched but the hoofs are *not* hot. The hindquarters are not swollen. The temperature varies and may be as high as 105 degrees Fahr. and even more. Horses which are good feeders, e.g., those which eat up some of their bedding, seem to be more predisposed, especially when in hard training. Treatment consists of a good saline purge followed after six hours or so by a soapy enema and only a little green food, the animal being kept warm in an airy box. Salicylates and bicarbonates as well as urotropine mixtures in fairly large doses are given every three hours. If the heart shows signs of weakness injections of camphor salicylate, 10 c.c. every three hours, may be

substituted for the above mixture. The ailment does not seem to spread from one horse to another. With the above line of treatment the animal apparently recovers completely within a week, usually after three days. It has been customary to follow the above treatment with a course of vaccine, the object being to avoid recurrence and roughening of joint surfaces. Three cases were injected intramuscularly with a proprietary rheumatic vaccine every three days commencing with 4 c.c., increasing by 1 c.c. until 10 c.c. were inoculated at the seventh and last dose. Trypan blue 1 per cent., 10 to 20 c.c., was given intravenously in conjunction with the vaccine in every case, but only on three occasions, as horses do not tolerate it well. The other four horses were inoculated with vaccine prepared by myself. The organisms were obtained from a London laboratory; they were hæmolytic streptococci, diplococci, and staph. aureus and albus, and all had been isolated from cases of arthritis. The organisms were grown in broth for three days at 37 degrees Cent.—filtered through a Seitz filter at room temperature at a pressure of 80 mm. Formalin (1 in 2,000) was added to the filtrate after making sure of purity and sterility by culture and examination of films.

To this filtrate and the proprietary vaccine the animals appeared to respond well.

(b) The sub-acute form is indicated by a short type of action when the horse is trotted immediately when brought out of the stable; after a short time the stride becomes more or less normal. The joints appear to be only very slightly tender. No abnormal temperature is shown. Two cases showed slight but distinct shoulder lameness.

The seven cases so affected responded to the vaccine prepared by me and trypan blue. (Trypan blue was given because some cases treated with vaccine alone improved but did not recover entirely.)

The two cases with shoulder lameness did not improve at all with vaccine and trypan blue treatment or following massage with counter-irritants. Both were thoroughbreds—a horse and a mare.

II. *Cairo S.P.C.A. Horses.*

Through the kind permission of Mr. A. Miller and the co-operation of the S.P.C.A. Senior Veterinary Surgeon, Dr. A. El-Shazly, I have collected specimens for the isolation of micro-organisms from arthritis cases for investigation and vaccine preparation.

(a) *Cases examined.*—Horses with swollen tender joints, or horses short in action with tender joints were selected; so far ten cases have been examined.

(b) *Samples collected.*—Under aseptic conditions, 10 c.c. of blood from the jugular vein was collected and 5 c.c. seeded on agar and on broth and incubated.

(c) Six cases gave negative results; from four cases the following organisms were recovered: *staphylococcus aureus of a non-hæmolytic type;* gram positive and streptococci in short chains of a *non-hæmolytic* nature; staphylococcus *albus* of a *non-hæmolytic* type.

Further work is in progress concerning the isolation of organisms and their pathogenicity to laboratory animals, and vaccine preparation and its use. Results will be reported in due course.

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Abstracts of Current Literature

Some Cases of Tuberculosis of the Vulva in Cows. By KURT ALLANSSON. *Skand. Veterinärtskr* (1941). *XXXI,* 14.

THE author describes three cases of tuberculosis of the vulva in cows. Two of the cases were examined in detail and were found to be primary infections. The tuberculous lesions were localised in the labia and the spura-mammary lymphatic gland. In one case the uterus, close to the cervix, was also involved. There was some evidence that in one of the cases the infection appeared to have begun as a wound infection following an injury to the vulva.

Nomenclature of Biological Products (Aggressins, Toxins and Toxoids).

It is quite common to hear veterinary surgeons in this country apply the term " serum " to products concerning the preparation of which neither antisera nor antitoxins form any part. *The Canadian Journal of Comparative Medicine* (1941) *5,* 1, refers to the misuse of the term serum and defines the terms aggressins, toxins and toxoids. Part of the article is reproduced below. A perusal of the extract will be found useful to veterinary surgeons and it is hoped will lead to the use of the appropriate terms to designate the different products.

" Aggressins are germ-free products obtained by expressing under pressure the juice from muscles of animals which have been destroyed in the last stages of an infection caused by a specific bacterial agent. The juices are filtered and a preservative added. The final product as distributed must be bacteriologically sterile. Specific aggressins, however, render bacteria more aggressive. This in conjunction with the fact that some time must elapse after inoculation before any immunity is established, should make it clear that the use of these products are contra-indicated in animals which are already infected or which may be exposed immediately, after inoculation, to the specific infection, against which the aggressin is prepared.

" Toxins are also germ-free products obtained by separating the fluid portion of liquid media; in which species of bacteria capable of producing toxins are cultivated. These products when unmodified by chemicals or heat are toxic for animals. Unmodified toxins are not used in veterinary medicine, but may be employed in conjunction with specific antitoxins to establish active immunity. Toxins modified and made harmless with formalin are termed toxoids. The products have recently come into use and reports of their efficacy as immunizing agents are excellent. Toxins should not be confused with other germ-free culture filtrates, which although similarly prepared are not true toxins, since they are harmless for healthy animals. Such products are mainly employed as diagnostic agents, classical examples of which are tuberculin and mallein."

Studies Relating to Brucella Abortus Infection. I. On the Occurrence of the Organism in the Blood Stream. II. On the Occurrence of the Organism in Serous Swellings or Hygromata on Cattle. By F. A. Humphreys and Thos. Moore. *Canadian Jl. Comp. Medicine* (1941). *5,* 5 and 11.

I. Some investigations have been carried out from time to time to show if *Br. abortus* can be recovered from the blood of infected cattle. In some cases direct blood cultures were made, whilst in others the blood was passaged in guinea-pigs from which cultures were made at the appropriate time. It is recognised that better results are obtained with blood from infected human

beings and laboratory animals than with infected cattle. Some workers have formed the opinion that a definite invasion of the blood can be demonstrated when agglutinins begin to appear. The above authors carried out a series of tests in which material containing *Br. abortus* was inoculated into guinea-pigs and they were able to recover the organism, by cultural methods, from the blood of 14 per cent. (12 out of 84). They also found that by sub-inoculation of blood into another series of guinea-pigs a similar percentage of positive cultural results was obtained. There was evidence that in some cases the blood of the guinea-pigs yielded positive results while attempts to culture the organism from the lesions in the spleen, etc., were unsuccessful. Concerning cattle, a series of 116 specimens of venous blood was cultured from naturally and artificially infected animals. In no case was the organism cultured. In some cases the specimens were taken immediately after the act of abortion, but even at this stage, when the presence of the organism in the blood might be expected, the results were negative.

II. The contents of 45 serous swellings (hygromata) were examined; 35 of the cattle reacted positively and six negatively to the agglutination test for the presence of *Br. abortus* antibodies. From 15 specimens *Br. abortus* was obtained in pure culture by direct cultural methods. In 25 of the swellings the inoculation of guinea-pigs with the contents resulted in the demonstration of the organism. By a combination of cultural and guinea-pig inoculation methods, 27 or 60 per cent. of the swellings were shown to be infected with *Br. abortus*. In one animal whose blood showed no agglutination titre and in which the fluid of the swelling did not agglutinate above 1 in 50, the presence of *Br. abortus* in the fluid was proved by guinea-pig inoculation. In two of the animals neither the blood nor the fluid of the swelling showed agglutinins; *Br. abortus* was isolated from the fluid of the hygromata of both animals by direct cultural methods. Taking all the data presented there was evidence of *Br. abortus* infection in about 93 per cent. of the animals that developed hygromata.

The Bacterial Flora of the Semen of Remount Stallions. By R. G. YAGER. *Vet. Bull. Supplement to the Army Medical Bulletin* (1941). *XXXV*, 33.

THE author examined 25 samples of semen over a period of two months. Each sample was collected from the urethra immediately after service. The results of bacteriological examination confirm the findings of other workers that all samples of semen contain bacteria. A list of nine different isolated organisms is given as well as a group which was not identified. In 16 per cent. of the specimens hæmolytic streptococci were isolated. The organism was not always present, as was shown by specimens taken from the same animal on different occasions. The author states that "Since *Streptococcus sp.* has

been incriminated as the etiological agent of numerous diseases of the genital tract and of the new-born, it is with this in mind that the writer believes that bacteriological examination of the stallion's semen and of the genital tract of mares is important, and such positive cases should not be bred until free of organisms that have pathogenic significance."

He did not find any cultures of *S. abortivo-equinus.*

Phenothiazine as an Anthelmintic for Cattle under Field Conditions. By L. E. SWANSON and B. E. CARLISLE. *Vet. Med.* (1941). *XXXVI,* 312.

THIS article is an account of the use of phenothiazine in seven herds of cattle, consisting of calves, yearlings and adult animals. Clinical descriptions of the infestations are given and in one herd (Herd No. 1) post-mortem work on four calves revealed heavy infestations with *Hæmonchus contortus, Trichostrongylus axei, Œsophagostomum radiatum, Bunostomum phlebotomum* and *Cooperia* spp. The animals were fasted for 18 to 24 hours before phenothiazine treatment was given but were allowed access to water. The drug was administered in hard gelatine capsules in the following doses: Calves three to four months—30 gm.; calves under 150 lbs.—40 gm.; calves 150 to 250 lbs.—50 gm.; calves 250 to 400 lbs.—60 gm.; yearlings and weak adults—70 gm.; adult cows, bulls and steers—80 gm.

From the work done the authors draw the following conclusions: Unconditioned phenothiazine as an anthelmintic was tested under farm conditions on 537 cattle of various ages and was found to be efficient and economical when used at the above dosage—all animals from three-months-old calves to adult cattle being treated. The drug was found to be comparatively non-toxic. (In Herd No. 2 some deaths occurred within three to 25 days following the treatment. The authors do not attribute the deaths to the effects of the drug.) The drug is easily administered in ½ to 1 ounce capsules and appears to be practical, as well as a highly efficacious treatment for cattle under farm and range conditions. The capsules should be lubricated to facilitate ease of swallowing. Single treatments have given results superior to frequent dosings with other anthelmintics. Animals to be treated should be fasted 18 to 24 hours and allowed access to water. Animals may be turned out to pasture or fed immediately following treatment.

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